

Supplementary Materials for  
**POU2AF2/C11orf53 functions as a coactivator of POU2F3 by maintaining  
chromatin accessibility and enhancer activity**

Aileen Patricia Szczepanski *et al.*

Corresponding author: Zibo Zhao, zibo.zhao@northwestern.edu; Lu Wang, lu.wang1@northwestern.edu

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**The PDF file includes:**

Figs. S1 to S6  
Legends for tables S1 to S6

**Other Supplementary Material for this manuscript includes the following:**

Tables S1 to S6

## Supplementary Figure

### Figure S1. Landscape of SCLC subtype-specific dependency

A) RNAseq TPM gene expression data for the 17 cell lines were retrieved from DepMap Public 21Q3 datasets. The Z-score heatmap showed the gene expression in the same order as Figure 1A.

### Figure S2. Identification of C11orf53 as a new SCLC-P subtype marker

A) The location of C11orf53 gene on chromosome 11q23.1. B) Comparison of protein produce of C11orf53 gene (1810046K07Rik in mouse and zgc: 158412 in zebrafish) between human, mouse and zebrafish by CLUSTALW. C) The aligned score for similarity between human C11orf53, mouse, and zebrafish zgc: 158412 was calculated by ClustalW sequence alignment. D) C11orf53 expression in 17 SCLC cell lines. Log (TPM+1) expression data were retrieved from DepMap Public 21Q3 dataset. E) Peptide blocking assay was performed to validate specificity of C11orf53 homemade polyclonal antibody. Recombinant antigen peptides (final concentration 100 µg/ml) were used as blocking peptides, BSA (100 µg/ml) was used as a negative control. F) The NCI-H526 cell line was transduced with either non-targeting CRISPR sgRNA, or two distinct C11orf53 specific sgRNAs, for three days, respectively. The cell cycle was determined by PI-staining and FACS analysis.

### Figure S3. C11orf53 regulates lineage-specific genes expression at super-enhancers

The average plot shows the co-localization of C11orf53 peaks detected by two antibodies (A), and the Pearson Correlation coefficient value was determined by using multiBigwigSummary and plotCorrelation functions from deepTools (B). C) The box plot shows the expression change of genes nearest to C11orf53 peaks in each of the four clusters. D) The pie plot shows the impact of

C11orf53 on super enhancer (SE) associated genes in NCI-H526 cells. E) Pathways analysis with genes nearest to Cluster 1 peaks of C11orf53. F) and G) The Venn-diagram shows the overlap of down-regulated gene (F) and up-regulated genes (G) in NCI-H526 cells treated with JQ1 (1  $\mu$ M) or two distinct C11orf53 sgRNAs, n=2. Pathway analysis by Metascope of 355 genes (Log2FC > 1) that are co-downregulated by JQ1 treatment or C11orf53 depletion (H), and 220 genes (Log2FC > 1) that are co-upregulated by JQ1 treatment or C11orf53 depletion (I). J) The representative tracks show the treatment of JQ1 also reduced the expression levels of C11orf53 targeted genes.

**Figure S4. Loss of C11orf53 reduces enhancer activity and chromatin accessibility**

A) The ATAC-seq was performed in NCI-H526 cells transduced with either non-targeting CRISPR sgRNA, or two distinct C11orf53 specific sgRNAs. The log2FC change of ATAC-seq signal was centered on C11orf53 peaks. The representative tracks show the ATAC-seq signal and active enhancer marks at PTGS1 gene loci (C11ORF53 binding) (B), and RAB1A gene loci (no C11orf53 binding) (C).

**Figure S5. Co-occupancy of C11orf53 and POU2F3 in SCLC-P cells.**

IP of endogenous POU2F3 from NCI-H526 cells followed by IB for POU2F3 and C11orf53 (A) and vice versa (B). C) Sorted and centered heatmaps generated from ChIP-seq data show the occupancy of C11orf53 and POU2F3 in NCI-H211 SCLC cells. All rows are centered on C11orf53 peaks. D) The average plot shows the enrichment of H3K4me1, H3K27ac, and POU2F3 at C11orf53 loci in NCI-H211 cell line. E) The representative tracks show the co-localization of POU2F3 and C11orf53 at *SOX9*, *GFI1B*, and *IRAG2* gene loci in NCI-H211 cell line. F) ChIP-qPCR was performed in NCI-H526 (F) and NCI-H211 (G) cell lines to determine the chromatin

co-occupancy of C11orf53 and POU2F3 at active enhancer gene loci of *SOX9*, *GFI1B*, and *IRAG2*. IgG was used as a negative control, n=3. Two-tailed unpaired student's *t*-test. \*\* $P < 0.01$ ; \* $P < 0.05$ .

**Figure S6. C11orf53 is recruited by POU2F3 to chromatin.**

A) HEK293T cells were transfected with GFP-tagged C11orf53 in the presence of either Halo-tag, or Halo-tagged POU2F3. The cellular fractionation was isolated and the protein levels of C11orf53 and POU2F3 were determined by GFP or Halo-tag antibodies. HSP90 was used as cytoplasmic protein control, and the histone H3 was used as nuclear insoluble protein control. B) The average plot shows the chromatin occupancy of C11orf53 in HEK293T cells transduced with C11orf53/Halo or C11orf53/Halo-POU2F3. The SCLC cell line NCI-H526 was transduced with either non-targeting CRISPR sgRNA, or two distinct POU2F3 specific sgRNAs for 72 hours, followed by cellular fractionation assay. The protein levels of POU2F3 and C11orf53 in each fraction was determined by western blot. HSP90 was used as cytoplasmic protein control, and the histone H3 was used as nuclear insoluble protein control. The chromatin recruitment of C11orf53 by POU2F3 at active enhancer gene loci of *SOX9* and *GFI1B* was determined by ChIP-qPCR, n=3. Two-tailed unpaired student's *t*-test. \*\* $P < 0.01$ ; \* $P < 0.05$ . E) The SCLC cell line NCI-H526 was transduced with either non-targeting CRISPR sgRNA, or two distinct C11orf53 specific sgRNAs, or two distinct POU2F3 specific sgRNAs for four days, respectively. The mRNA levels of tuft cell specific markers, including *ALOX5*, *SOX9*, *SUCNR1*, *NREP*, *PTGS1*, *GNG13*, *TAS1R3*, *GNAT3*, and *IRAG2* in each group was determined by RNA-seq, n=2, two-tailed unpaired Student's *t* test. \*\* $P < 0.01$ ; \* $P < 0.05$ .

## **Supplementary Table**

### **Supplementary Table 1.**

Essential genes for each SCLC subtype.

### **Supplementary Table 2.**

Differentially expressed genes in C11orf53 depleted cells.

### **Supplementary Table 3.**

Super Enhancer associated genes in NCI-H526 cells.

### **Supplementary Table 4.**

C11orf53 occupied genes in NCI-H526 cells.

### **Supplementary Table 5.**

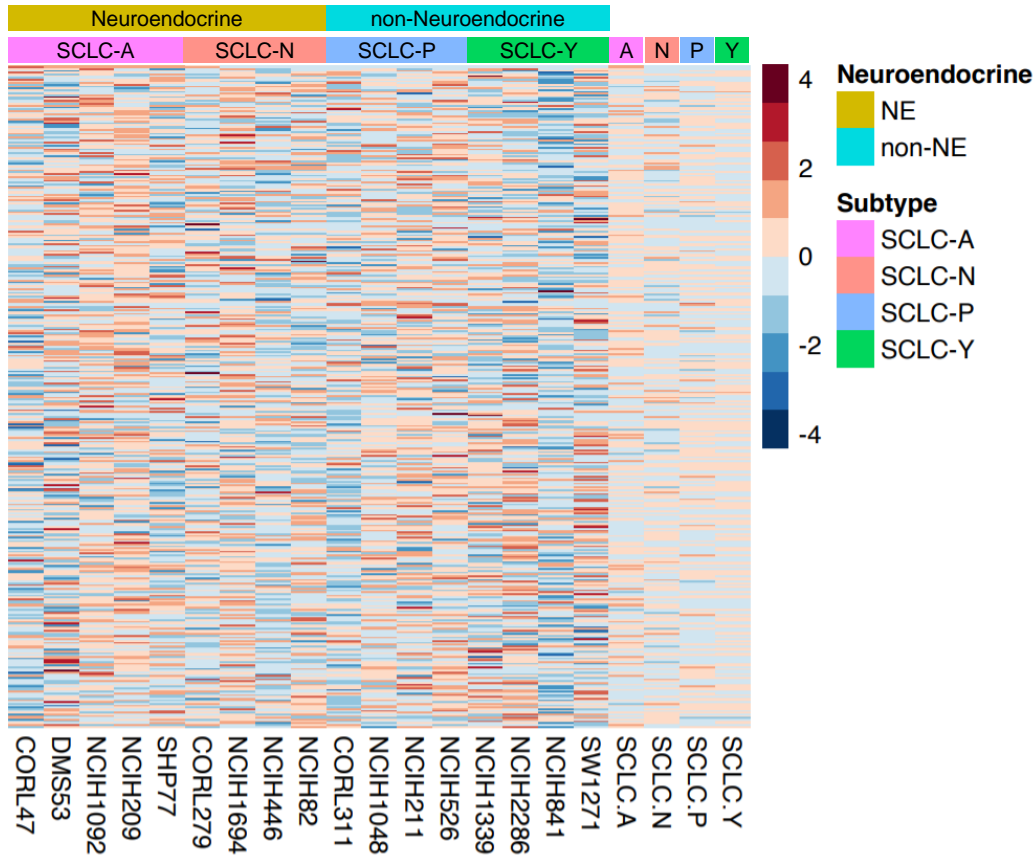
Differentially expressed genes upon JQ1 treatment in NCI-H526 cells.

### **Supplementary Table 6.**

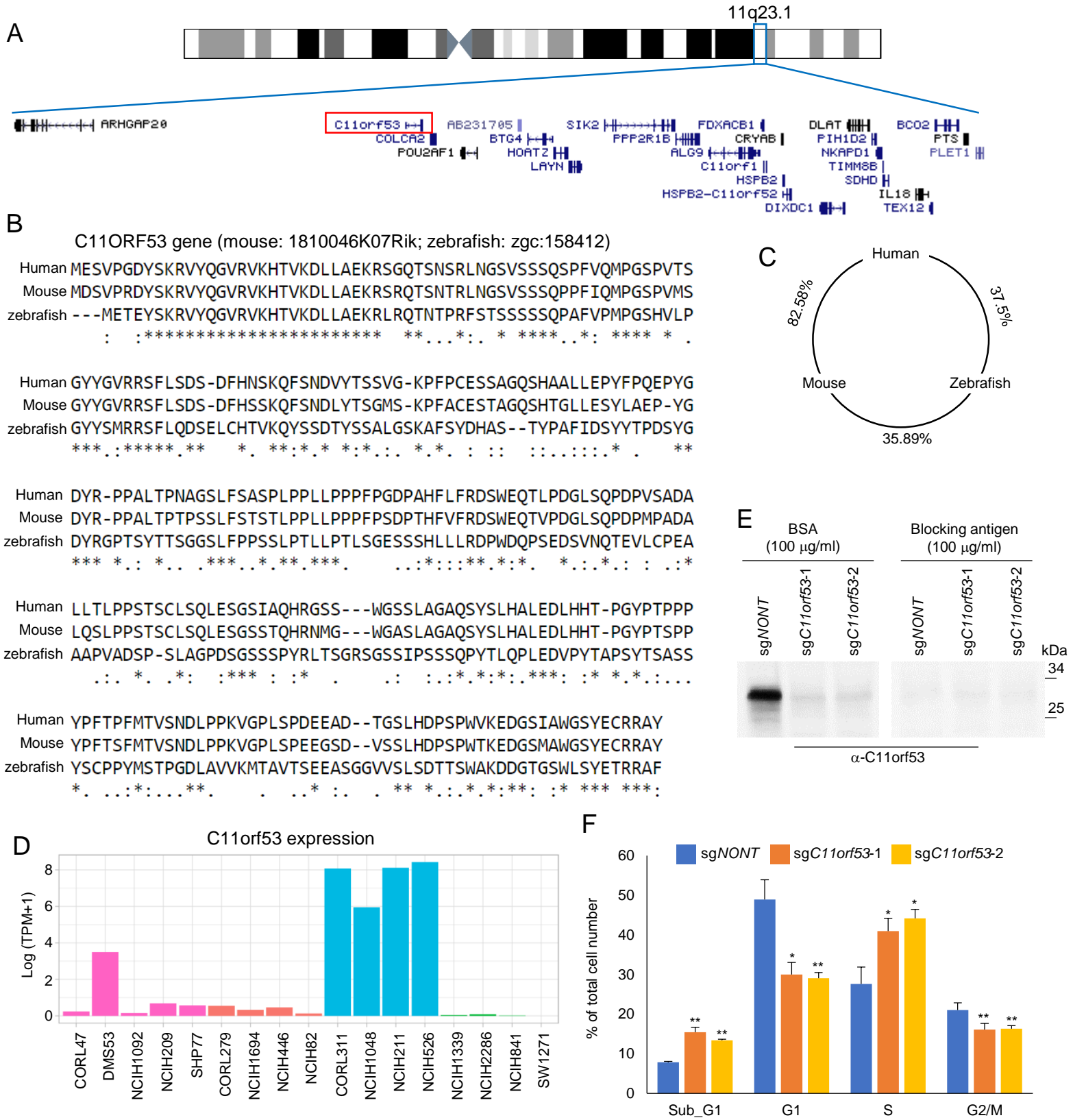
Differentially expressed genes in POU2F3 depleted cells

Figure S1. Landscape of SCLC subtype-specific dependency

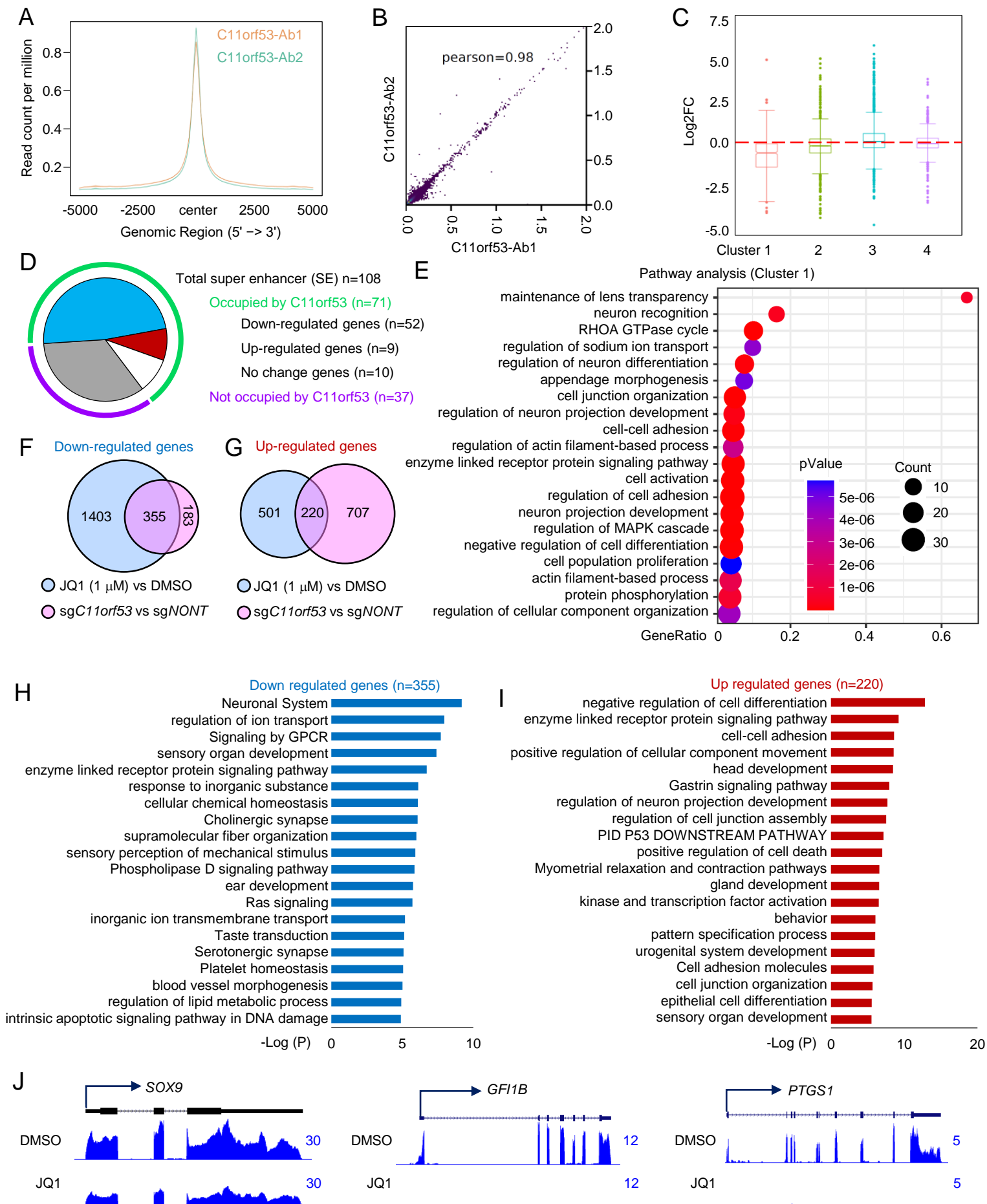
A



# Figure S2. Identification of C11orf53 as a new SCLC-P subtype marker

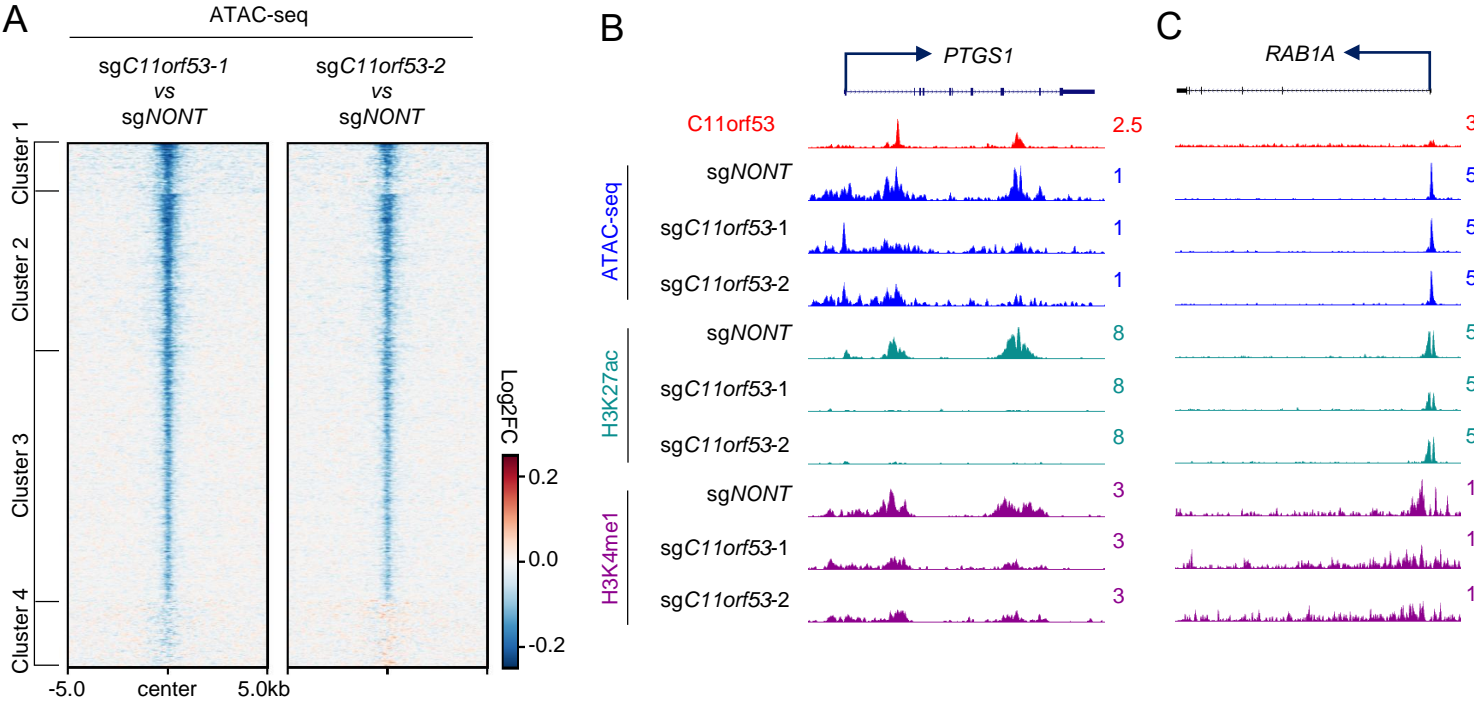


**Figure S3. C11orf53 regulates lineage-specific genes expression at super-enhancers**

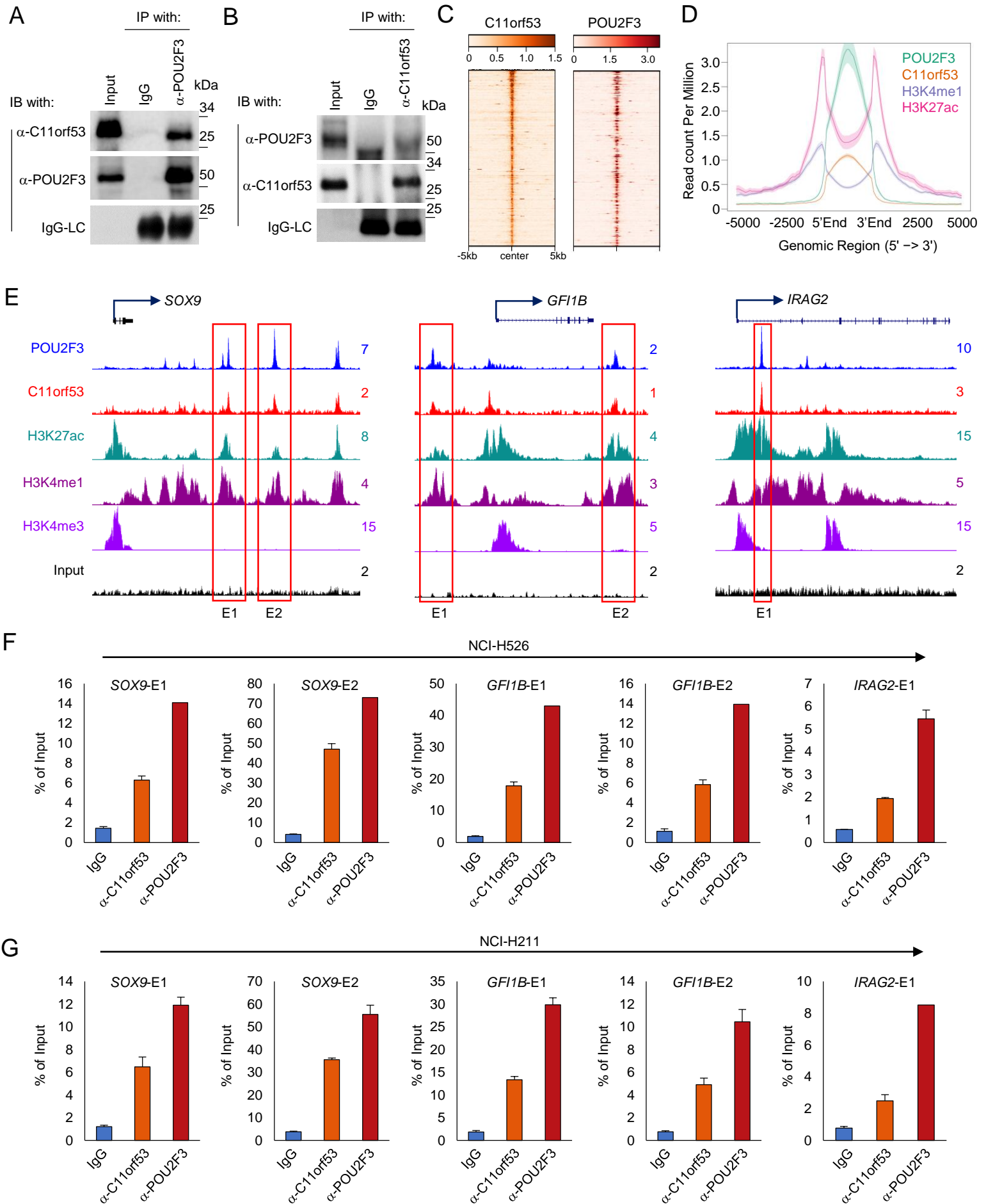




**Figure S4. Loss of C11orf53 reduces enhancer activity and chromatin accessibility**



**Figure S5. Figure S5. Co-occupancy of C11orf53 and POU2F3 in SCLC-P cells.**



**Figure S6. C11orf53 is recruited by POU2F3 to chromatin**

